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THE OVULE AND FEMALE GAMETOPHYTE OF DIOON.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXXVI.

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(WITH NINE FIGURES AND PLATES XIII–XV)

ONLY two of the nine genera of cycads have received any considerable attention from morphologists. In these two, Cycas and Zamia, the life history is fairly well known and many of its phases have been studied in great detail. In Stangeria the development of the sporangia has been investigated, and work on the remaining six genera is fragmentary.

Through the courtesy of the Botanical Society of America[1] the writer was enabled to visit the Mexican tropics for the purpose of securing material of Dioon and Ceratozamia. The hearty cooperation of Governor TEODORO A. DEHESA, who is an active educator as well as a statesman, made it possible to collect an abundance of material in a very short time. Mr. ALEXANDER M. GAW, of the State Bureau of Information, Xalapa, Mexico, had many collections of material brought into Xalapa from the field and forwarded to me after my return to Chicago. To both of these gentlemen I wish to express my sincere thanks, since the investigation would have been very limited without their efficient assistance. The photographs used were made by Dr. W. J. G. LAND.

The subject will be treated under the following heads: I. Dioon in the field; II. Material and methods; III. The ovule; IV. The female gametophyte; V. The microsporangium; VI.

[1] The grant was made in December 1904 by the Botanical Society, at the St. Louis meeting of the A. A. A. S., and was for the purpose of securing material for a morphological study of Dioon and Ceratozamia.

Male gametophyte; VII. Fertilization; VIII. The embryo and seedling. The first four are presented in the present paper; the remaining subjects have been investigated and the results will be published soon.

I. DIOON IN THE FIELD.

Dioon edule occurs in great abundance at Chavarrillo, about 25^{km} east of Xalapa, the capital of the State of Vera Cruz. During March 1904 I made frequent trips through this region and, besides the tedious work of preparing material, was able to make a few observations upon the plant as it occurs in the field. A second trip, devoted largely to field study of both Dioon and Ceratozamia, was made in September 1906. As the results of this trip will be published in a separate paper, only a brief description of the general appearance of Dioon is given at this time.

Associated with Dioon are occasional specimens of a large Opuntia. Orchids and Tillandsias are not infrequent on the trees at the bottom of ravines, though neither of these plants flourish on the rocky slope where Dioon is at its best. In the lower portions of ravines several species of Selaginella are abundant. Dioon is so abundant on the rocky slopes that from a favorable point of view as many as a hundred plants may be counted. Seedlings are numerous and one always meets these and then small crowns before coming upon plants large enough to bear cones. In spite of the seedlings, it is doubtful whether the limited range is being extended.

In habit Dioon resembles *Cycas revoluta* (*fig. 1*). Some call it the "Dolores palm;" but the natives are more likely to call it *Tio Tamal* ("Uncle Tamal"), because they use the large endosperm in making *tamales*. The largest plant measured had a trunk nearly 3^{m} in height, and plants $1–1.5^{m}$ in height are not infrequent. The trunk often rises obliquely, as shown in the figure, and as noted in taxonomic descriptions.

The age of individuals.—Even in the largest plants the leaf scars are perfectly distinct over the entire surface, so that it is possible to determine with almost absolute accuracy the total number of leaves which a plant has borne. Professor LUIS MURILLO has estimated the age of individual plants by noting the number of leaves in a crown, the duration of the crowns (two years), and the entire number of

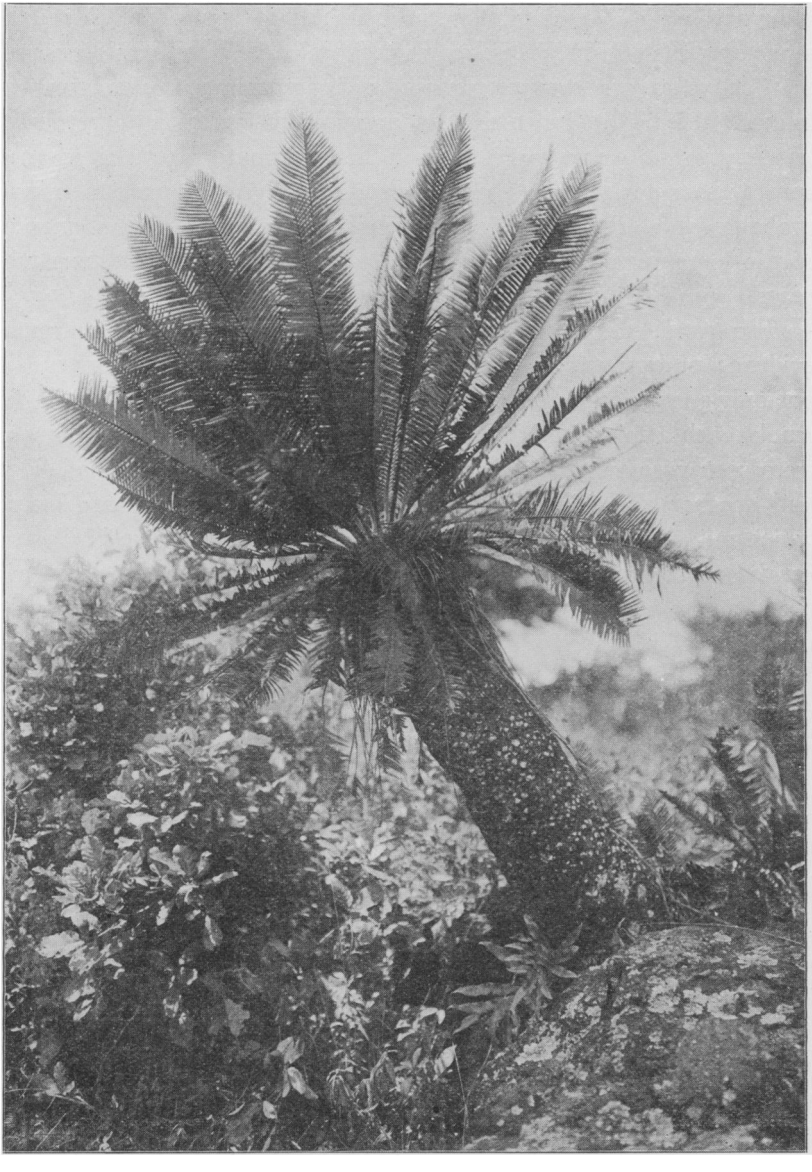

FIG. 1.—Ovulate plant of *Dioon edule* on rocky hillside at Chavarrillo, Mexico; the trunk is 1.5^m high.

leaf scars. From such data, the age of one plant with a trunk 1.45^{m} in height and 26^{cm} in diameter was estimated at 970 years. A small plant only 21^{cm} in height is known to have been in cultivation more than 40 years, and was presumably a fine specimen when brought in from the field. In cultivation a crown may persist more than two years, for the crown of a plant in the Washington Park (Chicago) conservatory has remained vigorous for at least five years. It seems probable that MURILLO'S estimate is conservative, and that many of the large plants have reached an age of more than a thousand years.

The trunk.—The trunk is always straight and shows no external evidence of branching; among the thousands of plants observed, one only showing definite branching. The specimen had a Y-shaped trunk, each arm of the Y bearing a large crown. A few specimens were seen with two, three, or four crowns; and one plant had five and another six. The extra crowns do not come from loose buds which might become detached, as is so commonly the case in *Cycas revoluta*, but are all interlocked at the top of the stem. Some of the extra crowns are probably due to injuries received in the removal of cones, while others originate from the germination of seeds which had not fallen to the ground but had remained in the nest of the crown. Buds like those of *Cycas revoluta* also occur; occasionally they are found near the bases of old trunks, and at the top they are quite common. Some of these buds have well-developed crowns and would doubtless grow into independent plants if they should become detached and gain a suitable foothold. Root tubercles were observed but they were infrequent.

The ovulate cones.—In 1904 fruiting plants were not abundant; among plants large enough to bear cones not more than one in ten was in fruit, and among these staminate plants were more numerous than ovulate. In 1906 at least one-third of the larger plants bore cones. I was informed that a plant fruits every other year, and judging from the proportion of plants bearing cones in 1906 this would seem true; but an estimate based upon the proportion of plants bearing cones in 1904 would make the interval very much longer. The ovulate cones are large and ovoid (*figs. 2, 3*). Cones weighing 5^{kg} are common; and one large cone weighed 6^{kg} after

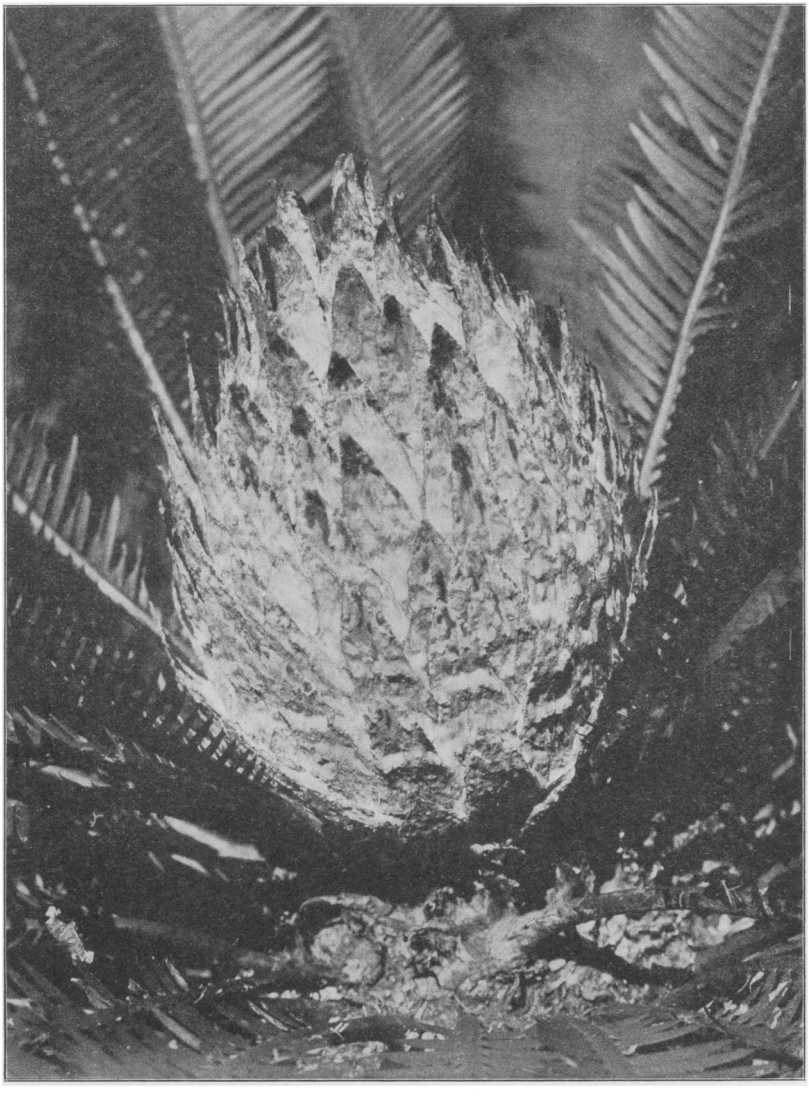

FIG. 2. —Ovulate cone 30^{cm} in length; some leaves have been trimmed away.

it arrived in Chicago, more than two weeks after it had been taken from the plant. This was a March cone and probably would have gained another kilo before June. The length of the mature

cone is 20–30^{cm}, and the greatest diameter is 12–20^{cm}. Young cones picked November 1, 1904, five months before the cones reach full size, weighed 1.5–2^{kg} after reaching Chicago. The single cone rests snugly on the plant at the center of the crown of leaves, the peduncle being entirely concealed, so that it is difficult to remove

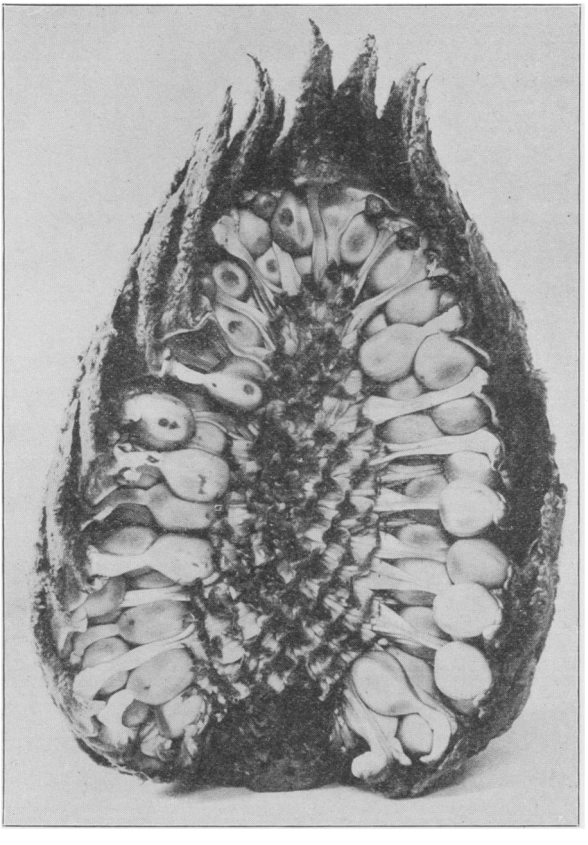

FIG. 3.—Ovulate cone 33^{cm} in length, showing position of ovules.

the cone with a knife. The easiest way is to grasp the cone with both hands and push it firmly to one side until the peduncle snaps with a clean transverse break. After the seeds are ripe the peduncle elongates somewhat, so that the cone often leans to one side, perhaps facilitating the dispersal of seeds. There is a well-developed abscis-

sion layer at the base of the petiole of the sporophyll, so that the whole sporophyll drops easily when the seeds are ripe. A second abscission layer, which separates the ovules from the sporophyll, does not mature until a much later period.

II. MATERIAL AND METHODS.

In March the ovulate cone has almost reached its full size, and the staminate cone of the same season has decayed. The first staminate cones were sent from the field on June 30, 1904. These showed the four microspores still held together by the wall of the spore mother cell. From this point to the discharge of the motile sperms a complete series in the development of the male gametophyte was secured. In the female gametophyte the series is complete from the appearance of the archegonium initial to the germination of the seed. The series showing the development of the embryo is very complete.

Nearly all the material was fixed in chrom-acetic acid in various proportions, with or without the addition of osmic acid. The following formula is excellent for the pollen tube structures and for young ovules: chromic acid, 1^{gm}; glacial acetic acid, 4^{cc}; 1 per cent osmic acid, 2^{cc}; water, 100^{cc}. This fluid will not penetrate the microsporangia, and with older ovules it not only causes some plasmolysis but makes the endosperm very hard to cut. After the endosperm has become starchy, better results were secured by using a slight modification of a formula suggested by Dr. LYNDS JONES: 50 per cent. alcohol, 100^{cc}; commercial formalin, 6^{cc}. This reagent penetrates well and fixes rapidly. Iron-alum haematoxylin stains brilliantly after it, but the safranin gentian-violet orange combination does not give as bright a stain as with material fixed in chromic solutions. After this reagent, the starchy endosperm is not so hard to cut. After the stony coat of the ovule has become hard, it is almost impossible to cut it with any knife without injuring the archegonia. For such stages the ovules were sawed in two transversely with a fine wire-like fret saw. The upper part of the endosperm with its archegonia was then trimmed into suitable shape for cutting. The extremely thin blade of the Gillette safety razor, soldered to any suitable handle, is particularly adapted for such trimming, since it

causes no damaging pressure as does the wedge-shaped blade of a scalpel or ordinary razor. The nucellus usually remains within the cup-like top of the ovule. Four strokes with a sharp scalpel will cut through the nucellus against the stony background and remove a piece containing the pollen tubes. Methods for studying the living sperms will be given in a second paper. For showing the grosser structures of the ovules, sections 4 or 5^{mm} in thickness were dehydrated and then cleared in xylol. For tracing the vascular bundles, the pseudo-stalks of ovules were cut under water and the bases were placed in eosin. The outer bundles soon become conspicuous on the surface, and the inner bundles are easily traced by removing the endosperm and scraping away the greater part of the inner fleshy layer of the integument.

Most of the sections were stained in safranin and gentian-violet; some were stained in iron-alum haemotoxylin. Magdala red and anilin blue proved to be a good combination, especially for pollen-tube structures, and it is quite convenient, since no clearing agent is necessary, the slides being taken directly from the absolute alcohol and mounted in Venetian turpentine. Overstaining in the Magdala red can be corrected, even after the cover glass is in place, by exposing the slide to direct sunlight for a short time.

III. MEGASPOROPHYLLS AND OVULES.

No other cycad, except Cycas, has such large and leaf-like megasporophylls as Dioon (*figs. 4–6*). Both the leaf-like character of the megasporophylls and their comparatively loose association in the cone are more suggestive of Cycas than of Zamia or Ceratozamia, which are the geographical neighbors of Dioon. The remaining occidental cycad, Microcycas, is too imperfectly described to allow any comparison of the cones.

In Dioon the sporophylls at the base of the ovulate cone are yellowish or greenish, with little hair except along the central portion of the back. There are four or five turns of the spiral of these sporophylls, followed by one or two turns of somewhat hairy sporophylls. The remaining sporophylls are densely covered with long brown hairs, the lower ones on the back and edges and a small portion of the upper part of the inner face, and the rest not only upon the back

and edges but upon the entire upper half of the inner face. This gives the whole cone a brown color and a densely hairy appearance. It would seem impossible for the cone to be wetted by rains. The changes in temperature in this region are so slight that the hairy condition could hardly be related to this factor.

The lower greenish sporophylls never bear ovules or even primordia of them. The next sporophylls have occasional primordia, but it is only when the larger sporophylls are reached that normal ovules

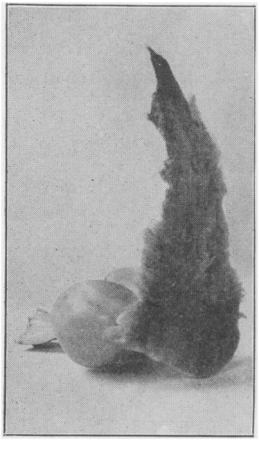

FIG. 4.—Ovulate sporophyll, back and side view. ×½.

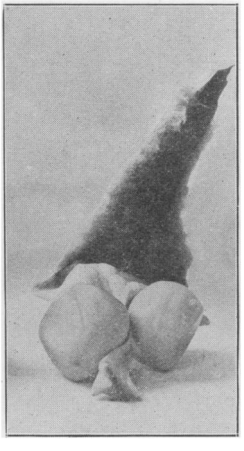

FIG. 5.—Ovulate sporophyll, inside view; ovules sessile. ×½.

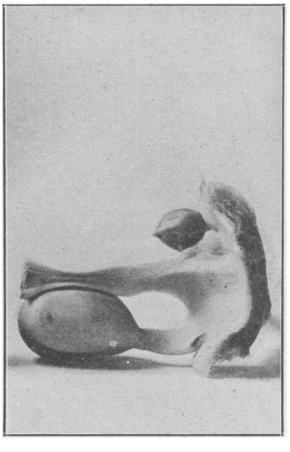

FIG. 6.—Ovulate sporophyll, abaxial side; one ovule with pseudo-stalk, the other sessile. ×½.

appear. Even the uppermost sporophylls bear ovules which sometimes ripen into seeds. The uppermost sporophyll is almost circular in transverse section. Its two ovules usually abort, as do those of the next sporophyll below it, but from this point, down to the sterile sporophylls at the base of the cone, each sporophyll bears two ovules which may develop into seeds. The ovules in a cone number about 100–300, and probably 200 ripe seeds is a liberal estimate for the larger cones. Where plants are isolated and pollination is uncertain, there are few ripe seeds, or even none at all. It would not be safe to say that the ovules of Dioon do not attain their full size unless pollination has taken place, for it is well known that the ovules of

other cycads reach their full size without the stimulus of pollination. However, the only greenhouse cone of Dioon which has come to my notice had only abortive ovules, and in the field the ovulate cones at any considerable distance from staminate plants are likely to contain only abortive ovules. Such ovules have not been pollinated. In cones bearing ripe seeds the abortive ovules have usually been pollinated, but have failed to develop from lack of room or of nutrition. It is easy to determine whether pollination has taken place, since the course of the pollen tubes is marked by conspicuous brown lines upon the nucellus.

The youngest ovules secured in 1904 were sent from the field November 1. These were 1^{cm} in length and showed the archegonium initials. Only one small cone had ovules as young as this, the ovules in other cones of this date measuring 1.5^{cm} in length and showing the central cell of the archegonium. November 14, six weeks after pollination, the average length of the ovules is about 2.3^{cm}; in the following spring (April 3), when the ovules had reached their full size, the largest ones measured 4^{cm} in length and 2.2^{cm} in diameter. The average length is about 3^{cm}, and the diameter 2^{cm}. Many ovules are nearly spherical, measuring about 3^{cm} in length and 2.8^{cm} in diameter. The ovules are perfectly smooth, and until nearly ripe are white, but become cream-colored or yellowish when exposed to the air; at maturity they have an orange color which contrasts sharply with the pale yellow of the naked portion of the sporophyll. They are sessile, but many of them appear to be stalked because strains due to the growth of the sporophylls and ovules draw out the base of the sporophyll into a stalk-like structure (*fig. 5*).

The ovule of Dioon reaches its full size before the stony layer becomes hard enough to occasion any serious difficulty in sectioning. Median longitudinal sections 3 or 4^{mm} in thickness, well dehydrated and cleared, but not stained, are best for a study of the general topography (*fig. 10*); while fresh ovules whose bundles have taken up eosin are most convenient for tracing the vascular system (*figs. 7, 8, 9*). The epidermis is smooth, strongly cutinized, and contains no stomata. At the base of the ovule the abscission layer is marked by a distinct constriction (*fig. 10, a*). The opening of the micropyle is amber or brownish in color from the drying of the pollination drop which,

judging from the caliber of the micropyle, must be quite large, although it was not observed directly. Early in November the various tissues of the ovule are recognizable, and early in December the layers of the integument are almost as distinct as in the following March, although the cells of the stony layer have not begun to thicken, and microtome sections of the entire ovule can still be cut. The general topography of the ovule, as it appears later in December, is shown in *fig. 10*. In this figure the endosperm and fleshy tissues are dotted, the stony layer is more deeply shaded, and vascular bundles are represented by dark lines. The integument conists of three layers,

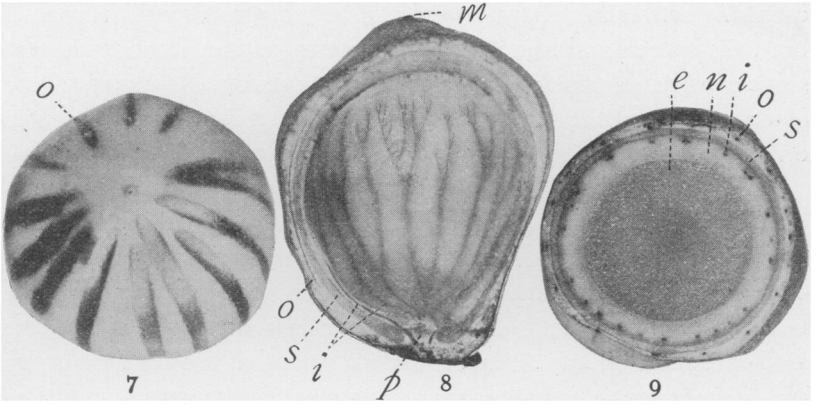

FIG. 7.—Ovule photographed from above; the eosin has spread and exaggerated the size of the outer bundles (*o*). ×2.

FIG. 8.—Inner vascular system of ovule, treated with eosin and photographed after the endosperm and part of the inner fleshy layer had been removed: *i*, bundles of inner vascular system; *m*, micropyle; *o*, bundle of outer vascular system; *p*, basal papilla; *s*, stony layer. ×2.

FIG. 9.—Transverse section of ovule treated with eosin: *e*, endosperm; *i*, bundle of inner vascular system; *n*, inner fleshy layer of integument and fused portion of nucellus; *o*, bundle of outer vascular system; *s*, stony layer. ×2.

an outer and an inner fleshy layer, with a stony layer between them. Only a small portion of the nucellus is free from the integument. The fleshy layers of the integument are comparatively simple in structure.

The outer fleshy layer.—The outer layer at the stage shown in *fig. 11* is not sharply marked off from the stony layer. These two layers

cannot be split apart, and even as late as May, when the stony layer has become very hard, the fleshy layer cannot be peeled off; it is only after it has become somewhat dry that it can be peeled off from the stony layer. However, the two layers easily separate as early as December if an ovule is cut in two and then allowed to decay for a few days in a damp atmosphere. The walls of the epidermal cells are considerably thickened and the outer surface is strongly cutinized. Most of the cells of the two layers just beneath the epidermis contain tannin, which occurs in only less abundance down to the level of the outer bundles. The cells containing tannin lie mostly in rows extending in the same general direction as the bundles. The large mucilage canals lie between the bundles and also have a general vertical course, but they branch and sometimes anastomose, so that some transverse sections of the canals are found even in longitudinal sections of the ovules. Beyond the zone of the bundles is a region of parenchyma cells (*fig. 11, p*).

The stony layer.—This layer, except at the deep pit which is occupied by the basal papilla, is thickest at the base and thinnest at the extreme apex. From the thin spot at the apex down to the lower part of the free portion of the nucellus it is thicker than throughout the middle two-thirds of the ovule. While the stony layer of the ripe seed is extremely hard, it is tougher and more elastic than the stony coat of most nuts, doubtless due to its complicated structure. The outer cells of the layer are small and isodiametric (*fig. 11, i*). In November they are not very sharply marked off from the inner cells of the outer fleshy layer; in fact, it is only after the walls of the stone cells have begun to thicken that the boundaries of the layers can be determined with accuracy. Beyond the small isodiametric cells is an irregular zone of cells elongated in a more or less longitudinal direction (*fig. 11, e*); then follows a zone of similar cells elongated transversely (*fig. 11, et*); and finally another zone of cells considerably elongated longitudinally (*fig. 11, i*). As growth continues, the small outer isodiametric cells simply increase in size and their walls became thickened. In the cells of the other three regions there is not only an increase in size and a thickening of the walls, but various displacements occur, so that the structure in March is much more complicated than in the preceding November. The elongating cells become

crowded and interlaced and sometimes even branch, thus giving rise to an extremely tenacious tissue,

The inner fleshy layer.—This layer contains the inner vascular system, but otherwise consists of rather uniform, slightly elongated parenchyma cells. The cells are smaller than those of the main body of the nucellus, but there is no definite boundary between them. The boundary between the inner fleshy layer and the stony layer is less indefinite, and as early as January the two layers may be peeled apart, though with a rather uneven break. In small November ovules the inner fleshy layer has about the same thickness as the stony layer, but from this time the fleshy layer grows more rapidly, and in early December ovules is much thicker than the stony layer (*fig. 10*). The rapidly growing endosperm then begins to encroach upon the adjacent cells, which we regard as nucellar tissue intimately united with the inner fleshy layer of the integument, although it must be admitted that the ontogeny shows no indication of such a union. The encroachment continues until in the ripe seed all the tissues between the endosperm and the stony layer is reduced to a thin dry membrane, which peels off easily and cleanly, and shows very clearly the distribution of the inner vascular system. The inner fleshy layer is to be regarded as belonging to the integument rather than to the nucellus, because it is continuous with the inner fleshy layer of the free portion of the integument; because the bundles which it contains often extend into the inner fleshy layer of the free portion of the integument but never into the free portion of the nucellus; because in half-ripe ovules the fused portion of the nucellus may be peeled away from the layer containing the inner bundles; and, still more important, because such a conclusion is warranted by a comparison of the cycad ovule with those of fossil gymnosperms.

The phylogeny of the three layers.—This is an interesting question. Has the single complex integument always been single, or does it represent two integuments which have become fused? This is a question to which no definite answer can be given, because the early development of the ovule has never received sufficiently careful study in any cycad. The early stages in the development of the integument are passed before the cone breaks through the scale leaves, and consequently no material is likely to be secured in greenhouses. In the

field, where material may be abundant, it would be necessary to cut out the growing points of many plants to secure a few ovulate cones. And even then, judging from a slight examination of the ovule of Ginkgo, the integument of which also has two fleshy layers with a stony layer between them, we should be likely to find the integument arising as a single undifferentiated tissue. A study of the integument of Dioon after its various tissues have become somewhat differentiated also fails to give any definite evidence as to its single or double nature.

I have been able to examine the integuments of several other cycads, but even where the differentiation between the layers is sharp, as in *Zamia integrifolia*, where the outer fleshy layer in both its cell-structure and cell-contents is sharply marked off from the stony layer, and where the differentiation between the stony layer and the inner fleshy layer is also rather distinct, there is no satisfactory evidence that the integument has ever been anything but a single structure. In Ceratozamia the layers are even less defined than in Dioon.

Judging from the literature of the subject, especially from the work of Miss STOPES (**16**), who has made the most thorough investigation, no study of the integument of living cycads can yield conclusive evidence as to its single or double nature. However, a comparison of the cycad ovule with that of the fossil Lagenostoma lends much strong support to the theory that the cycad integument is a double structure. OLIVER and SCOTT[13] suggest that the cupule of Lagenostoma is equivalent to the outer fleshy layer of the cycad integument, while the canopy of a Lagenostoma may have become simplified into the stony layer of the cycad seed. Miss STOPES' opinion is indicated by the title of her recent paper "On the double nature of the cycadean integument."[16] She believes that the plane of fusion between the two coats is either between the inner and outer portion of the stony layer or between the stony layer and the inner fleshy layer. The outer fleshy layer and the outer portion of the stony layer she believes to be too intimately connected to be separated morphologically. The structure of the Dioon integument, as it appears in sections, would seem to indicate that the plane of union has been between the inner and outer layers of the stone (*fig. 11, et, i*). The integument of Ceratozamia would bear a similar interpretation; but in *Zamia integrifolia* the outer fleshy layer is so sharply marked off from the

stony layer that one could easily regard the former as an adnate cupule equivalent to the free cupule of Lagenostoma. In spite of the close morphological continuity between the stone and the outer flesh during the early development of the integument of most cycads, the two layers separate readily at maturity. The carpels of syncarpous ovaries of angiosperms become so closely united that the planes of fusion seem completely obliterated and the tissues appear perfectly continuous, but at maturity the carpels separate along the original planes of union. Hence the close continuity of the tissues should not exclude the view that the outer fleshy layer of the cycad ovule represents a structure which has become adnate to the stony layer. On the other hand, the ready separation at maturity must not be urged too strongly as an argument in favor of a union at this plane, because at maturity the inner fleshy layer of all the cycads also separates just as readily from the stony layer, and the inner fleshy layer of the integument never separates at all from the lower portion of the nucellus. While it must be confessed that the cycadean integument itself offers no conclusive evidence of a double nature, we agree with Miss STOPES that it possibly represents a double structure. In regard to the plane of fusion we could not agree with her, but think it more probable that the union has taken place between the outer flesh and the stone.

The nucellus.—The nucellus is free from the integument only for a short distance, the free portion extending little beyond the top of the endosperm. The free surface is cutinized and the epidermal cells contain tannin. When very thick sections are cleared in xylol, the free portion is limited by a very conspicuous black line. The sharp beak closes and hardens immediately after pollination, imprisoning the pollen grains in the pollen chamber. After pollination the upper portion in the surface of the nucellus soon becomes marked by brown lines caused by the haustoria of the pollen, which never penetrate deeply, but lie just beneath the epidermis. In December a considerable mass of tissue separates the pollen chamber from the top of the endosperm, in which the archegonial chamber has not begun to form. Subsequent growth of the endosperm, together with extensive disorganization of nucellar tissue and also some enlargement of the pollen chamber itself, finally destroys all tissues between the pollen tubes and the archegonia. The mode of disorganization which results in the

formation of the pollen chamber is easily seen. The middle lamella softens and breaks down, thus setting free a few cells which are soon resorbed, and so forming the beginning of a pollen chamber. The subsequent encroachment of the disintegrating region is most rapid downward, but is also increasingly extensive at the periphery, so that the completed chamber is more or less funnel-shaped.

Below the free portion of the nucellus its most conspicuous feature is the thick jacket which surrounds the endosperm (*fig. 11, ej*). This jacket is sharply differentiated from the rest of the nucellus, and is so tenacious that it can be stripped off with forceps. The megaspore-membrane sometimes adheres to it, but more often clings to the endosperm. Although the material contained no early stages in the formation of the jacket, it is evident that it originates as a layer only one cell in thickness. Where cells of the layer do not divide periclinally, it remains only one cell thick; if there is a single periclinal division, the layer becomes two cells thick; while another division would make it three cells in thickness. In the archegonial region a considerable portion of the cells undergo one periclinal division; while in the chalazal region nearly all the cells show such a division, and a large number undergo a second periclinal division. On the sides there is a gradation between these two extremes. The less prominent and less complete jackets of the Coniferales have been described by THOMSON (**21**).

The cells of the jacket are very frequently binucleate and occasionally three nuclei are found. In comparison with the size of the cell these nuclei are rather large, but their chromatin content is very scanty and the nucleoli are small (*fig. 12*). It is not at all uncommon in these cells to find a complete chromatic spirem lying perfectly free in the cytoplasm, with no trace of a nuclear membrane or any achromatic structures (*fig. 13*). In such cases the nucleolus is always present and is somewhat larger than that of resting nuclei. The amount of chromatin in the spirem is vastly greater than in the resting nuclei. This unique condition does not lead to the formation of daughter nuclei or even to the reorganization of the single nucleus, and the significance of the phenomenon is not evident. The cells of the jacket at this time are centers of extreme metabolic activity, all the materials for the nutrition of the rapidly growing endosperm

passing through them, and in passing undergoing some change before reaching the endosperm. It may be that the peculiar nuclear condition is brought about by the extreme activity of the jacket cells.

The walls of the jacket cells are suberized and stain deeply with safranin, thus contrasting sharply with the cellulose walls on both sides of them. The most conspicuous cell contents are irregular granules and coarse strands, which stain so deeply with safranin that in a section of a November ovule the jacket appears as a bright red circle easily visible to the naked eye. The material of the granules appears very much like that of the outer part of the megaspore membrane, which THOMSON (**21**) found to be a suberized structure. He described similar granules in other cycads and found them to consist of amylodextrin, but in his form the amylodextrin disappeared before the stage represented in *fig. 13* was reached. (There are no pits in any of the cells of the jacket.) The irregular granules and strands adhere closely to the cell wall even after the cytoplasm has been drawn away by reagents, as shown in *figs. 12* and *13*. No granules or strands are found on the outer walls of the jacket cells or in the cells on either side of the jacket layer. The endosperm jacket differs decidedly from the archegonial jacket in having no pits in the walls; consequently, all substances must pass through the jacket by the usual method of transferring material from one cell to another.

The jacket is doubtless concerned with the nutrition of the structures within, like the jacket about the embryo sac of an angiosperm, the jacket about the seeds of gymnosperms, or the tapetum about the sporogenous cells of microsporangia. While the jacket in all these cases is concerned in nutrition, the mode of nutrition and the nature of it are different. It is most active while the endosperm is still spherical. As the endosperm elongates and approaches its full size, the jacket disintegrates, and at the stage shown in *fig. 10* it has broken up and only suberized fragments remain. As to its morphological nature, the jacket corresponds to the jacket or tapetum which surrounds the sporogenous tissue of microsporangia. In a microsporangium the jacket surrounds a large number of spores, while in the case under discussion it surrounds only one spore, which, at the stage shown in *fig. 10*, has developed an extensive prothallium. LANG (**12**), who secured early stages of this structure in Stangeria, describes an

extensive sporogenous tissue in the ovule, and believes that the outer portion of it gives rise to the tapetum, while all the tissue within except the functional megaspore disappears. Unfortunately, no such early stages were secured in Dioon, but since the jacket surrounding the microspores may originate from either sporogenous or sterile cells, there seems to be no objection to regarding the jacket surrounding the megaspore as equivalent to that surrounding the microspores, especially since homosporous forms, to which all heterosporous forms must ultimately trace their origin, have well-developed jackets.

Base of the ovule.—The lower limit of the ovule is marked superficially by a slight constriction as soon as the abscission region begins to develop (*fig. 10, a*). This region does not extend straight across, but is depressed in the center, with the depression away from the body of the ovule. When the ovule breaks off, the slight protuberance at the base is due to the shape of the abscission zone. This zone, about twelve cells in thickness, consists of nearly isodiametric cells, and thus contrasts rather sharply with the elongated cells above and below (*fig. 14*). Almost as soon as the zone can be distinguished, intercellular spaces appear, which increase in size until they sometimes become as large as the cells themselves, making them appear somewhat like the stellate cells of Typha, Scirpus, and similar forms. These cells are rich in starch. Above the abscission region the axis of the ovule extends upward and terminates in a conspicuous projection which occupies a deep pit in the stony layer. This projection, which was described as the *Einstülpung* by Miss STOPES (**15**), may be called the basal papilla (*fig. 10, p*). In the unripe seed the tissue of the basal papilla is noticeably tougher than that of other portions of the outer fleshy covering. In the ripe dry seed it looks for a time like a toothpick broken off in the stony layer, but it soon decays like the rest of the fleshy layer and its place is marked by a conspicuous pit about 2^{mm} in diameter and extending almost through the stone. Large elongated cells containing tannin are abundant on both sides of the abscission zone, but they do not extend into it. Several large mucilage ducts, which lie within the region occupied by the vascular bundles, reach almost to the top of the basal papilla. In the position and shape of the abscission layer and also in the appearance of the basal papilla, Dioon resembles Lagenostoma as described by OLIVER

and SCOTT (**13**) and illustrated in their *fig. 26*. The rest of the tissue on each side of the abscission zone consists of large, elongated cells, forming a loose parenchyma with irregular intercellular spaces like those of the abscission zone, except that the spaces are larger.

Vascular system of the ovule.—The distribution of the vascular system is easily traced by cutting off the petiole of the sporophyll under water and placing the cut end in an aqueous solution of eosin. The vessels become filled with the solution in a few minutes. For studying the course of the bundles in the ovule itself, it is better to cut the ovule just below the abscission layer. While the fluid does not always penetrate the ultimate tracheids, the course of the bundles is sharply marked, as may be seen in *figs. 7–9*, which are photographs of ovules treated in this way. For studying the transverse distribution of bundles, series may be obtained very rapidly by cutting sections of ovules treated with eosin and simply placing the sections in order upon a sheet of paper. The abundant mucilage causes them to adhere firmly, and the bundles appear as bright red dots.

A transverse section of the petiole of the megasporophyll near the axis of the cone shows about seven bundles, arranged in a straight line. Two of these bundles, one at each end of the row, are usually larger than the others, and develop into the vascular systems of the ovules. The other bundles branch repeatedly, so that in the widest part of the lamina of the sporophyll a transverse section shows about thirty bundles, still arranged in one straight line. The outer bundle forks once as it passes to the ovule, so that a transverse section just below the abscission line of the ovule shows two bundles. From each of these two bundles a branch passes toward the outer fleshy layer of the integument, and another branch toward the inner fleshy layer, thus giving rise to an outer and an inner vascular system with the stony layer lying between them (*fig. 10*).

The bundles which supply the outer fleshy layer of the integument branch several times before they reach the level of lower limit of the stony layer, but from this point they extend to the micropyle with no branching at all (*fig. 7*). The number of these bundles in vigorous ovules varies from 10 to 17, with 14 as the most frequent number; in abortive ovules 12 and 13 are the most frequent numbers. In every cone there are some abortive ovules, and it may be that an

inferior vascular supply has something to do with their failure to develop.

The bundles passing to the inner fleshy layer begin to branch a little higher up than do those of the outer layer. There is some branching in the basal papilla; after passing out to the inner fleshy layer there is repeated forking, as shown in *fig. 8*, so that a transverse section taken at the level of the archegonia shows 40 to 60 bundles. After passing from the stony layer, these bundles run in the outer part of the inner fleshy layer of the integument and extend up to the free portion of the nucellus. Occasionally a bundle continues in the inner fleshy layer beyond the beginning of the free portion of the nucellus, but in no case was a bundle found entering the nucellus itself. Occasionally a small bundle passes through the middle of the basal papilla, through the thin portion of the stony layer, and into the tissue at the base of the endosperm. The development of the bundles and the significance of the various details of the vascular system have not been attempted.

IV. THE FEMALE GAMETOPHYTE.

Stages showing the origin and early development of the female gametophyte were not secured. Since fruiting plants are comparatively rare, and since they cannot be distinguished from vegetative specimens until the cones are well started in their development, the earliest stages could be obtained only by a vandal-like mutilation of plants large enough to bear cones. The youngest ovules, collected early in November, were about 8^{mm} in length, and showed the archegonium initials. The gametophyte had become cellular throughout, no free nuclear portion remaining at the center. Only one small cone had ovules as young as this, the ovules in the cones of this date measuring about 1^{cm} in length and showing the central cell of the archegonium.

During November, December, and January the pressure exerted by the young gametophytes is remarkable. At a slight cut into the tough layer which is to become stony, a distinct snap can be heard, the cut gapes open, and the gametophyte protrudes. Later, when its cell walls have become firmer, the gametophyte retains its form when cuts are made into the stony layer. When an ovule in which the

gametophyte is still spherical is cut through the middle, the gametophyte, as seen with the naked eye, has a beautiful, radiating appearance, looking somewhat like a transverse section of a twig with very fine and numerous medullary rays. Until late in December the endosperm is almost transparent, so that the young archegonia are easily seen and counted, even when the endosperm is removed entire. Ordinary newspaper print can be read through a section of endosperm 4^{mm} in thickness. At this stage the endosperm tastes sweet on account of the abundant sugar, but as soon as starch begins to form it takes on a translucent white color, and as the starch becomes abundant an opaque white. Even before the starch begins to appear, the endosperm commences to elongate, and in February has reached a length of 15^{mm} and a breadth of 10^{mm}. In April, when the endosperm has reached its full size, it is about 25^{mm} in length and 17^{mm} in breadth.

Early in December there not only is no archegonial chamber, but the top of the gametophyte containing the young archegonia is even elevated (*fig. 15*). In February the tissue at the rim of the elevation begins to grow rapidly, while the growth of the elevation itself is checked; consequently, the elevation soon becomes the bottom of the archegonial chamber (*fig. 16*). At the time of fertilization the archegonial chamber has reached a depth of 1 to 1.5^{mm} (*fig. 17*).

The megaspore membrane.—Early in November, while the endosperm is still spherical, the megaspore membrane is well developed. In fresh material one might easily mistake the endosperm jacket for the membrane, but the jacket is much coarser and can be stripped off entire with forceps. The membrane is comparatively delicate, but pieces several millimeters in length may be stripped off with forceps. When the nucellus is removed, the portion of the membrane covering the archegonia usually adheres to the nucellus rather than to the endosperm. The membrane is thinner at the apex and base of the endosperm than on the sides. In young ovules, with the endosperm still spherical, the greatest thickness of the membrane which was measured was 3μ at the apex, 3.1μ at the base, and 4.5μ at the sides. The average at this stage is about 3μ at the base and apex, and 4.1μ at the sides. In ovules whose endosperm has reached a length of 1.5^{cm}, but in which the archegonial chamber has not yet begun to form, the membrane is rather uniformly about 5μ in thickness. In germinating

seeds in which the embryo is beginning to break the stony layer, the membrane reaches a thickness of 9–10μ, but the membrane is homogeneous, there being no differentiation into layers as in earlier stages.

The membrane clearly consists of two layers, which may be called the endospore and exospore (*figs. 12, 18*). The outer layer in young ovules is three or four times as thick as the inner, but in older ovules the difference is not so great. The inner layer seems perfectly homogeneous under the highest magnification. The outer layer, under moderate magnification, looks somewhat like the dense outer layer of the megaspore membrane of Marsilea; but with a Bausch and Lomb $\frac{1}{16}$ immersion and Zeiss oc. 12 the structure appears to be comparatively loose (*fig. 18*). The entire outer layer consists of club-shaped bodies. In other gymnosperms similar bodies have been described as prismatic, but in Dioon they really consist of a globular or ovoidal outer portion connected with the inner layer of the membrane by a stalk. In surface view these bodies are seen to be regularly arranged and to be quite uniform in size, the diameter of the head in November ovules being about 0.85 μ, and in the following February about 1μ. As will be seen from the figure, the stalks form a comparatively open structure between the heads and the endospore. This open region appears as a nearly black line when seen in sections more than 1 or 2μ in thickness, so that there seem to be three layers, the extra layer being only an optical effect caused by the stalk region.

The membrane covers the entire female gametophyte, but after the pollen chamber breaks through the base of the nucellus the portion of the membrane covering the archegonial chamber is ruptured, so that the two chambers form one continuous cavity. This cavity is moist, but is not filled with a liquid. Only a few chemical tests were made and the results agreed with those of THOMSON (**21**), who found the outer layer of the membrane to be suberized, while the inner layer iss uberized only where it is in contact with the outer layer. The inner portion of the inner layer, next the gametophyte, consists of cellulose. That the megaspore membrane is a vestige surviving from an ancestry which shed the megaspores seems too probable to be questioned. If it could be assumed that the ancestors of all the living cycads had megaspore membranes of equal thickness, and that in the surviving forms the membrane has been reduced uniformly in all genera, then

the living genus with the thinnest membrane could be regarded as the farthest removed from its ancestry, and the genus with the thickest membrane would represent most nearly the ancestral condition. In spite of the uncertainty of such suppositions, it seems reasonable to regard a thick membrane as a primitive character, and while not conclusive evidence in itself, it deserves to be considered with other features in any discussion of the phylogeny of the cycads.

It would be interesting to know the thickness of the megaspore membranes of the other cycads at various stages in the life history. THOMSON (**21**) found that in *Cycas revoluta* the membrane is slightly more than 5μ in thickness at the stage when cell division is beginning in the endosperm. At a somewhat later stage the same writer found the membrane of *Stangeria paradoxa* to be $4.5\ \mu$ in thickness, and the same thickness is given for the membrane of mature seeds of *Zamia integrifolia;* while in *Ceratozamia longifolia* the membrane is 4.5μ in thickness at a stage when the archegonia have been formed. In *Dioon imbricatum*,[2] before the appearance of archegonia, the thickness was 3.8μ. I have measured the membrane in the above genera, except Stangeria, and find only such slight variations from THOMSON'S figures as may be accounted for by slight differences in stages of development. The extreme thickness of the membrane in the germinating seed of Dioon so much exceeds any of THOMSON'S measurements that it would be interesting to know whether the membranes of other gymnosperms increase so much in thickness when the seeds germinate. The megaspore membrane of Dioon is as thick as that of any cycad, and consequently, so far as this one character is concerned, Dioon is as primitive as any member of the group.

Development of the endosperm.—Early in November, about two months after pollination, the endosperm has become cellular throughout. If one might hazard a guess at previous stages, the November condition looks as if the free nuclear condition had been succeeded by the formation of very large cells which had been divided repeatedly. At a stage when the archegonium initial is first distinguishable, the peripheral cells of the endosperm about half way between the archegonial and chalazal regions present the appearance shown in *fig. 19*.

[2] This form is described by MIQUEL, in his *Prodromus Systematis Cycadearum* (1861), under *Dioon edule* Lindl., as *β imbricatum*, and is based upon leaf characters.

Except at its apex, the structure of the gametophyte is like that shown in this figure, a single layer of small peripheral cells being succeeded by layers of larger and larger cells. At the apex, where the archegonial initials are appearing, the single layer of small cells broadens rapidly into a lenticular group, from 7–10 cells in thickness. This group consists of about 200 cells, which are considerably smaller than those of the peripheral layer, as may be seen by comparing *figs. 19* and *23*, which are drawn to the same scale. The archegonium initials may be seen at the periphery of this group. At this stage, the cells of the gametophyte contain no starch or other food stuffs, the only visible contents being the nucleus, a scanty amount of cytoplasm, and the transparent cell sap.

Three weeks later, when the endosperm has reached a length of 15^{mm}, the cell contents seem to be just the same as before, no accumulation of foodstuffs being visible. Cell division has progressed rapidly, so that the row of four cells shown in *fig. 19, a*, is now represented by the two rows (sixteen cells) of *fig. 20, a*. Sections of the endosperm 4^{mm} thick, fixed in Flemming's solution early in December, appear somewhat darker in the region of the archegonia. In the chalazal region there is also a slightly darker color. Microtome sections show that the color is due in part to occasional grains of starch, but more particularly to small globules, probably oil, which stain black with osmic acid. The fact that the cells are considerably smaller in the archegonial region also makes the endosperm appear denser at this place. In the ripe seed the gametophyte shows considerable differentiation (*fig. 21*). The cells of the peripheral layer are small, rich in protoplasm, and contain numerous very minute starch grains. This layer contrasts sharply with the next, the cells of which are much larger and contain larger starch grains. This second layer is in turn fairly well marked off from the rest of the gametophyte, which consists of still larger cells densely packed with large starch grains. Numerous isolated cells containing tannin form a broad zone midway between the center and periphery of the endosperm. The tannin is much more abundant in the upper part of the gametophyte.

Development of archegonia.—Probably all of the superficial cells of the group at the apex of the endosperm are potentially archegonium initials. The number of archegonia varies from none at

all to ten. Several ovules at the period of fertilization showed no archegonial chamber and no trace of archegonia. Ten archegonia were observed in only a single instance, which was also exceptional in having two archegonial chambers. A single archegonium with a small archegonial chamber was observed in a few cases. Usually there are three, four, or five archegonia, with four as the most frequent number. An archegonium initial, which can be seen early in November, becomes distinguishable by its slightly greater size (*figs. 22, 23*). The division into a neck cell and central cell takes place early, probably in November (*fig. 24*). The neck cell divides almost immediately, forming the two-celled neck which is a constant feature in all cycads yet investigated. All December ovules not only showed the neck, but the neck had already divided into its two characteristic cells (*fig. 25*).

The central cell enlarges very rapidly, its scanty protoplasm forming a delicate layer pressed against the wall by the single large sap vacuole (*figs. 24, 25*). Even at the stage shown in *fig. 24*, the cells bordering upon the central cell are rather regularly arranged, and in *fig. 25* there seems to be a definite jacket. While this layer functions more or less in the nutrition of the central cell, the real archegonial jacket with its characteristic cell contents is formed later by both periclinal and anticlinal divisions in the jacket-like layer of *fig. 25*. The differentiation of the jacket is not so rapid at the apex of the central cell as at the sides and base. As soon as the jacket begins to be differentiated from the neighboring cells, the cytoplasm of the central cell increases rapidly, and soon the space which had been occupied by the single large vacuole is filled. The rapidly increasing cytoplasm shows a beautiful foam structure (*fig. 26*). At this stage there is no reticulum or even any fibers. The vacuoles of the central cell are much smaller at the apex and at its periphery than nearer the center. The walls of these vacuoles are themselves vacuoles, and there is a perfect gradation, so far as size and appearance is concerned, from the large vacuole near the center to the smallest vacuoles of the thin plates of cytoplasm which form the walls of the large vacuoles. Some of the walls of the larger vacuoles are shown in surface view in *fig. 26*, though most of the walls show only the edges, as in case of cell walls. The smallest of the vacuoles are small enough to come within BÜTSCHLI'S

limit for the size of meshes of genuine protoplasmic structure. The central cell of the archegonium is formed early in November, a little more than two months after pollination. The mitotic division which gives rise to the ventral canal nucleus and the nucleus of the egg takes place about the middle of the following May, so that the growth of the central cell and its nucleus extends over a period of six months. Throughout this entire period the growth is uniform, there being no cessation or retardation as would be the case in a colder climate.

The nucleus of the archegonium initial begins to enlarge very early, and is noticeably larger than the nuclei of the surrounding cells as soon as the initial itself becomes distinguishable (*fig. 22*); but while the growth of the nucleus continues steadily, it does not keep pace with the more rapid growth of the central cell. In March, after five months growth, the diameter of the nucleus has increased from 10μ to 70μ. The nucleolus, which at first was inconspicuous, has become prominent, and the chromatin granules are evenly distributed throughout the nucleus. At this time several large nucleolus-like bodies appear in the cytoplasm near the nucleus (*figs. 27, 29*). These bodies strongly resemble those which are found between the blepharoplast and nucleus in the body cell of Gingko, as described by HIRASE (9). At first they are solid and perfectly homogeneous; but as the nucleus of the central cell begins to divide, they become vacuolate (*fig. 29*), and before the mitosis has reached the metaphase they have broken up into innumerable small globules and granules (*fig. 30*). These bodies stain black with iron haematoxylin, and with the safranin gentian-violet combination they usually take the violet even when nucleoli are staining red with the safranin. Their exact nature and function was not determined, but they do not seem to differ, except in size, from the globules of nutritive material which are soon afterward brought into all parts of the egg by the haustoria.

The ventral canal nucleus.—While the growth of the nucleus of the central cell extends over a period of more than six months, its division, when once begun, is extremely rapid. The only figures found were in material sent from Xalapa on April 1, 1905, and fixed at Chicago on April 13 and 17. No figures were found in any of the numerous collections fixed in March. Some ovules fixed later than April 17, and even as late as May 13, showed the nucleus of the central cell

still undivided; but such ovules were from cones picked about the middle of March, and the ovules finally decayed without any division of this nucleus. The top of the typical archegonium in such material is shown in *fig. 28*, which is from a cone picked March 18, the material being fixed May 13, nearly two months later. The two neck cells are in perfect condition, but the central cell has begun to degenerate.

In normal material the spirem is broad but very tenuous in consistency. In the only cases noted it had evidently suffered from reagents (*fig. 29*). The shortening, condensation, and segmentation of the spirem were not observed, the next available stage being shown in *fig. 30*, where the segmentation into chromosomes has taken place. The splitting of the chromosome in the equatorial plate is shown in *fig. 31*. In this preparation the number of chromosomes was determined with reasonable though not absolute certainty to be twelve. In a late anaphase the chromosomes, while still retaining the U-shape, are becoming irregularly moniliform, looking as if they might break up into small pieces (*fig. 32*). Only a few stages in the formation of the spindle were observed. The first indication of it is a granular and fibrillar appearance which is more marked at the lower pole of the nucleus (*fig. 29*). There are no centrosomes, and in *fig.* 30 the poles are rather blunt. Fibers like the spindle fibers are abundant in the cytoplasm of the papillate projection in which the figure lies. In *fig. 31* the spindle is more sharply bipolar, and long mantle fibers are more conspicuous, although many of the spindle fibers are still continuous from pole to pole. *Fig. 32* shows no trace of the granules which mark the beginning of a cell plate, and later stages make it certain that no wall is formed between the daughter nuclei. The ventral canal nucleus remains free in the cytoplasm of the egg, as shown in *figs. 33–35*.

A ventral canal cell in cycads was first described by STRASBURGER (**18**) in 1876 for *Cycas sphaerica*. The next year WARMING (**23**) described one in *Ceratozamia robusta*, but soon concluded that he had been mistaken. TREUB (**22**) in 1884 failed to find any ventral canal cell in *Cycas circinalis*, and from that time it was generally believed that the cycads have no ventral cell. However, in 1898 IKENO (**10**) made an unmistakable demonstration of the critical mitosis in *Cycas revoluta*. At that time no ventral canal nucleus not separated from the egg

nucleus by a wall had yet been observed, and he merely followed current terminology. The term ventral canal nucleus was introduced by COKER (3) in 1902 to describe the condition in Podocarpus. IKENO'S figures show that no wall is formed between the ventral canal nucleus and that of the egg. WEBBER (24) soon afterward reported that in Zamia "a small cell is cut off at the apex of the archegonium," but here too the language is unfortunate, for no cell is cut off, and the ventral canal nucleus remains in the general cytoplasm of the egg, as shown by COULTER and CHAMBERLAIN (5). I have recently made preparations of Encephalartos showing the same condition. It is probable that no ventral canal cell is cut off in any of the cycads, there being merely a nuclear division.

It cannot be doubted that this represents an advanced stage in the reduction of the archegonium. It offers no exception to the method by which the row of neck canal cells of the bryophytes and pteridophytes has been reduced. In these groups binucleate neck canal cells are frequent. This means that the formation of a cell wall has failed to follow the nuclear division. The next stage in reduction would be the suppression of the mitosis, and thus a diminution in the number of neck canal cells. In this way the neck canal cells gradually become reduced in number, some pteridophytes showing only a single one. In gymnosperms there is no neck canal cell at all, and the ventral canal cell is being eliminated by the same process. In some genera, like Pinus, the ventral canal cell is separated from the egg by a wall. In other genera the nuclear division takes place, sometimes with a series of granules on the spindle indicating a rudimentary cell plate, but no wall is formed. In Torreya it seems likely that even the ventral canal nucleus is suppressed. The absence of a ventral canal nucleus has been reported for several genera, but the evidence is not conclusive. Dioon, Zamia, Cycas, and Encephalartos still preserve the mitosis, although the wall is no longer formed. In this particular, although the oldest of living gymnosperms, the cycads do not show as primitive a condition as do Pinus and some other Coniferales.

After the mitosis described above, the ventral canal nucleus forms a membrane and may enlarge slightly, but it soon disorganizes, so that at the time of fertilization a scarcely recognizable vestige remains.

Typical views are shown in *figs. 33, 33a,* and *34.* Occasionally there is considerable enlargement (*fig. 35*), as is often the case in Pinus. I have a preparation of Encephalartos which not only shows a reorganization and enlargement of the ventral canal nucleus, but the nucleus has moved down from the papillate projection toward the egg, suggesting the possibility of fertilization of the egg by the ventral canal nucleus. In STRASBURGER'S account (**33**) of fertilization in *Picea vulgaris* and in COULTER'S account (**3**) in *Pinus Laricio,* describing fusing nuclei of equal size, a large ventral canal nucleus was doubtless mistaken for a male nucleus, but that these were actual cases of fertilization of the egg by its own ventral canal nucleus there can be but little doubt.

The egg and archegonial jacket.—The egg of Dioon is the largest yet known in plants. It is seldom less than 4^{mm} in length and often reaches a length of 5^{mm}; the largest egg measured was 6^{mm} in length. The actual size, just after the formation of the ventral canal nucleus, is shown in *fig. 17a.* The egg at the right in this figure is shown again in *fig. 17,* which is magnified six diameters so as to show the comparative size of the papilla, ventral canal nucleus, and egg nucleus. Before fertilization the egg nucleus becomes much larger and more deeply placed than is represented in this figure. In *fig. 15,* which shows the actual size of the central cell before the beginning of the archegonial chamber, and in *fig. 16,* showing the beginning of the archegonial chamber, the nucleus of the central cell is too small to be represented even by a single dot.

The nutrition of the egg is practically the nutrition of the central cell, for it reaches its mature character before the mitosis which separates the ventral canal nucleus from that of the egg. GOROSCHANKIN (**8**) in 1883 described a continuity of protoplasm between the jacket cells and egg of Ceratozamia. He believed that strands of protoplasm pass through sieve plates in the pits of the jacket. IKENO (**10**) in 1898 made a more detailed study of the growth of the egg in Cycas. He also described protoplasmic continuity between the jacket cells and the egg, and a passage of proteid materials from the jacket into the egg. Miss ISABEL SMITH (**14**), who studied the nutrition of the egg in *Zamia floridana,* found no connecting strands of protoplasm, but found projections, which she called haustoria,

extending from the egg into the jacket cells. In Dioon there are haustoria similar to those of Zamia. An examination of Ceratozamia, Cycas, and Encephalartos showed that they also have haustoria like those of Zamia and Dioon. The origin, development, and function of the haustoria, together with changes in the jacket cells of the surrounding tissue, would be a long problem, involving chemistry as well as morphology.

During the early stages of its growth (*figs. 24–26*) the central cell receives food material from the surrounding cells by the usual method of transferring substances from one cell to another. Up to the stage shown in *fig. 25*, the wall of the central cell is very thin, with no visible pits; but later the inner surface of the wall undergoes an extensive secondary thickening, interrupted only by the large pits which are such a conspicuous feature of cycad eggs. After the haustoria have become fully developed, their cytoplasm is in direct contact with that of the jacket cells, so that substances may pass from the jacket cells into the haustoria as readily as from one part of the jacket cell to another. A paper was received from Drs. STOPES and FUJII (**17**) just as this account is going to press, describing sieve plates as found by GOROSCHANKIN (**8**). Their figures are evidently drawn from young material, in which the haustoria have not reached their full development. That there is protoplasmic continuity here, as elsewhere, we do not doubt; but if the pit-closing membrane persists in stages like those shown in our *figs. 37–41*, it has escaped our observation. The haustoria project far into the cells of the jacket, and the mere thrust may have ruptured the membrane. At the stage shown in *fig. 36*, it is possible that the membrane may still be present; but in stages like *figs. 37–41* we could not find any membrane, even after a reexamination of our preparations. Consequently, we see no reason for modifying the following account, which was written before the paper by Drs. STOPES and FUJII was received.

The general method by which nutritive materials reach the egg is easily understood. The tissue of the female gametophyte is filled with starch and other food materials, which in a changed form pass into the archegonial jacket and thence into the egg. The cells of the archegonial jacket at certain times contain numerous starch grains, much smaller than those in the surrounding endosperm cells, but

the starch is soon dissolved. The change from starch to a soluble form doubtless takes place repeatedly, because the starch at various stages in the development of the archegonium is sometimes present and sometimes absent. The protoplasm of the jacket cells is abundant and their nuclei are much larger than those of the surrounding tissue. The changes taking place within these cells and nuclei resemble those which occur in glandular cells, there being a period of accumulation, followed by discharge, and then exhaustion, after which the processes are repeated. Glandular activity begins in December, as soon as the archegonial jacket becomes distinguishable. At this time the protoplasm of the central cell consists of a thin peripheral layer, and still thinner lamellae which divide the interior into vacuoles of various sizes, those near the center being the largest (*fig. 36*). The activity increases gradually up to the time of fertilization, about the first of May, and then diminishes. At the close of the period of free nuclear division in the proembryo, the jacket is not very vigorous, but there is still some activity. Toward the close of the intrasporal development of the proembryo, the jacket cells become weak in contents and begin to break down; and after the embryo has broken through the base of the egg and advanced 4 or 5^{mm} into the endosperm, the jacket is scarcely recognizable. During the period of repose or exhaustion (*fig. 36*) the protoplasm of the jacket cells is finely vacuolated. The nucleus has a somewhat homogeneous, finely granular structure, in which the chromatin is not conspicuous, although it can be seen that most of it is in the half of the nucleus nearest the egg. The protoplasm of the haustoria is evenly granular.

As activity begins (*fig. 37*), the protoplasm of the jacket cells becomes more coarsely vacuolated and food materials of various shapes and sizes appear within it. The nucleus is particularly active. The nucleolus becomes saturated with a substance which stains black with iron haematoxylin, and the chromatin first becomes conspicuous and then obscured by a substance which also stains black and may be the same as that in the nucleolus. Material passes from the nucleus into the cytoplasm and from the cytoplasm into the haustoria. Once within the haustoria, the food materials are already within the egg. In passing from the haustoria to the deeper portions of the egg, the materials break up into smaller and smaller granules

and globules, so that the periphery of the egg always contains the most conspicuous contribution from the jacket. The food materials never take the form of "proteid vacuoles" as in Pinus, and are never in danger of being mistaken for nuclear structures. The appearance of the jacket and the periphery after a discharge is almost completed is shown in *fig. 38*.

The three preceding illustrations (*figs. 36–38*) show the conditions in young eggs while the protoplasm is still quite scanty, most of the space being occupied by large vacuoles. Later stages, after the egg has become filled with protoplasm and the large vacuoles have disappeared, are shown in *figs. 39–41*. In *fig. 39* the ends of the haustoria are covered by a frothy substance, and in *fig. 40* it is seen that this substance is passing into the egg. A large vacuole marks the place which has just before been occupied by frothy substance. The nucleus, as is often the case, is concave on the side next the haustoria. A later stage is shown in *fig. 41*, which represents various kinds of food materials within the egg. Globules and droplets of various forms are most abundant, but crystalloids are not infrequent. The crystalloids are cubical or may approach the spherical form, but are never fusiform like those so characteristic of the egg of Zamia. Starch is sometimes present but generally absent.

The structure of the protoplasm of the egg undergoes great changes, especially during the last two months of its development. In December the protoplasm, containing one large vacuole, forms only a thin layer pressed against the periphery of the central cell (*fig. 25*). In March the protoplasm has increased greatly in quantity and numerous vacuoles have appeared (*figs. 36–38*). In these figures, and even in considerably later stages, the lines represent the edges of lamellae, and the appearance is a strong argument in favor of the foam or *Waben* theory of the structure of protoplasm. In later stages, however, the foam structure disappears, and the protoplasm seems to be almost entirely in the form of fibrillae (*fig. 41*). The change seems due in great part to the breaking down of lamellae, thus leaving fibrillae at the junctions.

Why the nucleus of the central cell remains at the apex for so many months, and why after the mitosis the egg nucleus moves down

while the ventral canal nucleus remains at the apex, are questions still unanswered. A moment's reflection will convince any one that neither temperature, light, nor gravity has any appreciable influence. The path by which nutrition reaches the egg may be an important factor, for the extensive vascular system of the ovule terminates near the top of the endosperm. On the other hand, the same position of the nucleus is found in gymnosperms whose ovules have scarcely any vascular tissue at all. Later, during the formation of the proembryo, the polarity is reversed, the nuclear activity being most vigorous at the base of the egg. At this time it is evident that nearly all the nutrition is coming from the endosperm at the base of the egg. As a working hypothesis it may be suggested that the early position of the nucleus at the apex, and also the reversal of the polarity, are due to chemotaxis, the source of nutrition being the controling factor.

The egg nucleus.—The egg nucleus of Dioon is the largest which has yet been found in plants. Its usual diameter is about 500 μ, but nuclei sometimes reach a diameter of 600 μ. Not infrequently the nucleus is elongated, and in such cases its bulk is likely to be greater than that of the usual spherical nucleus. One nucleus measured 1475 μ by 380 μ. In spite of the immense size of this nucleus, its structures could not be interpreted. Its chromatin content is shown at the lower end of the spindle in *fig. 32*. Stages immediately following this were not secured, and in stages like *figs. 34* and *35* the chromatin can no longer be identified. From this time to the entrance of the sperm into the egg, the internal structure of the nucleus is shown in *figs. 42–44*, which represent details as they appear under a magnification of 1300 diameters. The entire nucleus, drawn to this scale, would be more than 6^m in diameter! In all parts of the nucleus is found a delicate network of varying thickness, and upon it or imbedded in it are granules and globules of various sizes. The smallest granules, which are almost always associated with the network or clinging to the surface of larger globules, stain with gentian-violet. Most of the larger globules stain with safranin, so that the general tone of the network is red; but some stain with the gentian-violet and in others the two stains blend. There are always a few which do not stain at all. In most preparations the network stains very faintly or not at all. The nucleoli, which are large and vacuolated, can usually be distin-

guished from the globules represented in *figs. 42–44*. If any of these granules represent the chromatin, the individuality of the chromosomes would appear to be hopelessly lost. No satisfactory study of the living nucleus was made. A nucleolus is visible in living material, but otherwise the contents seem nearly homogeneous. There are few globules and the network could not be identified. It is possible that most of the globules and the network are coagulation artifacts due to fixing.

The development of the egg nucleus seems to be essentially the same in all gymnosperms. Among the investigators who have studied this nucleus in various gymnosperms may be mentioned STRASBURGER (**19**, **20**), IKENO (**10**), BLACKMAN (**1**), CHAMBERLAIN (**2**), FERGUSON (**7**), and LAND (**11**). BLACKMAN, CHAMBERLAIN, and FERGUSON attempted to follow the behavior of the chromatin, but the accounts are inadequate, there being stages in which all fail to identify convincingly the chromatin. Miss FERGUSON'S beautiful figures of Pinus show the familiar structures with great accuracy, but just what structures are chromatin is not clear. My own series of Pinus is less complete, and those of other writers are still more incomplete. In Dioon it is evident that this nucleus behaves much as in Pinus. The solution of the problem is not easily attained in cycads on account of the inaccessibility of material and the difficult sectioning. In Pinus, with its abundant material and easy technique, a close series in the development of the nucleus and in the formation of the spirem at the time of fertilization would probably lead to an understanding of the egg nucleus of the whole group.

SUMMARY.

1. Dioon occurs in abundance at Chavarrillo, Mexico.

2. It is probable that plants often reach an age of more than one thousand years.

3. The ovulate strobilus is more like the loose ovulate strobilus of Cycas than the compact ovulate strobili of the other genera.

4. The megasporophylls are more leaf-like than those of any other genus of cycads except Cycas.

5. The integument consists of three layers: an outer and an inner fleshy layer, with a stony layer between them. The integu-

ment is probably double in nature, the outer fleshy layer representing the outer integument.

6. Only a small portion of the nucellus is free from the integument.

7. One vascular bundle passes from the sporophyll toward each ovule. Before entering the ovule the bundle forks, one branch forming the continuously branching system of the inner fleshy layer of the integument, and the other forming the slightly branched system of the outer fleshy layer.

8. The megaspore membrane varies in thickness from 3–4.5 μ in young ovules to 9–10 μ in mature seeds.

9. The number of archegonia varies from one to ten, with four and five as the most frequent numbers. The archegonium initial appears in October; the division into neck and central cell takes place almost immediately after; the mitosis which forms the ventral canal nucleus and egg nucleus takes place during the next May.

10. The central cell and egg during earlier stages receive food substances by the ordinary method of nutrition, but later receive food material through haustorial projections from the egg which are in direct contact with the cytoplasm of the jacket cells.

11. There are twelve chromosomes in the egg nucleus, which is the largest one yet known in plants.

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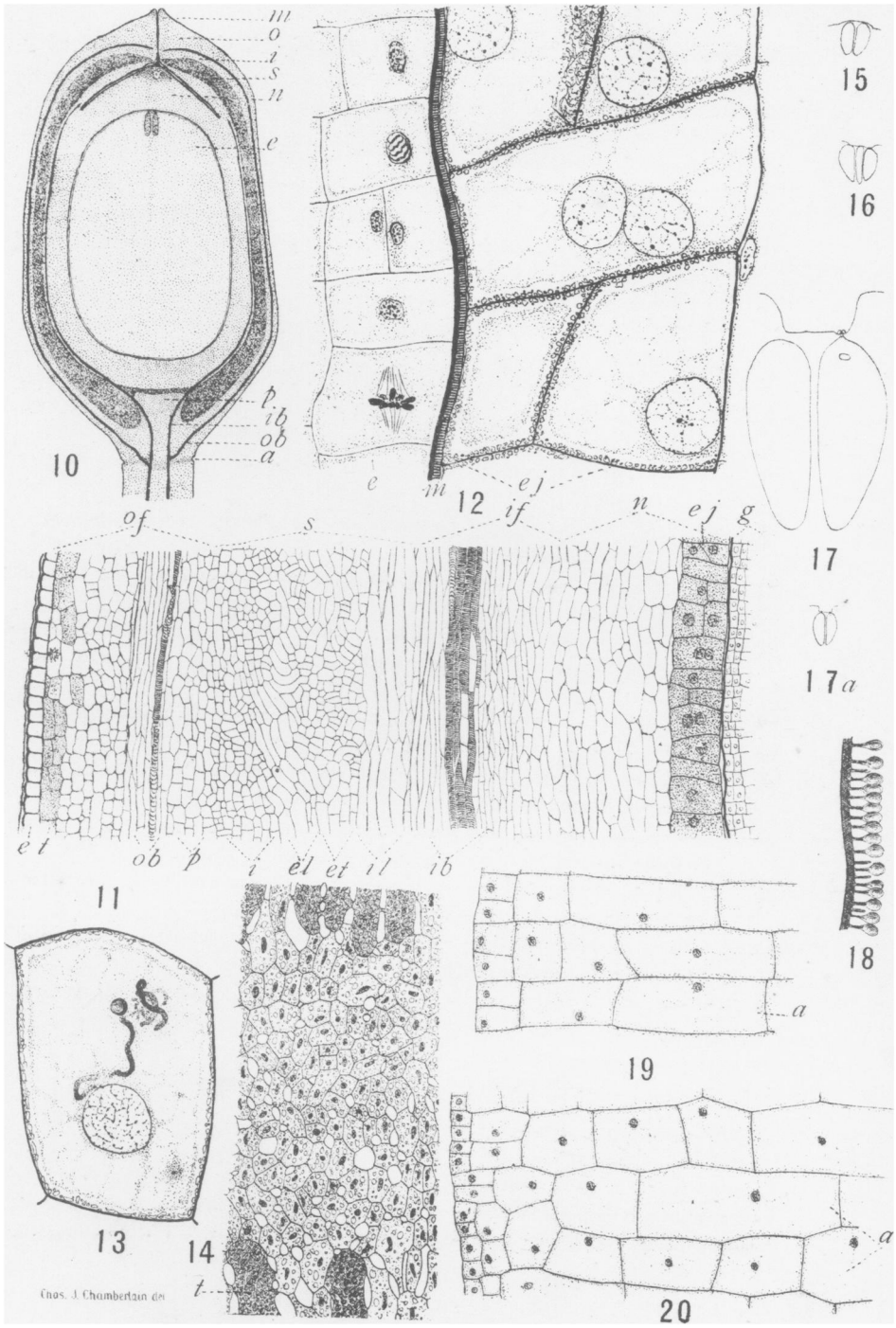
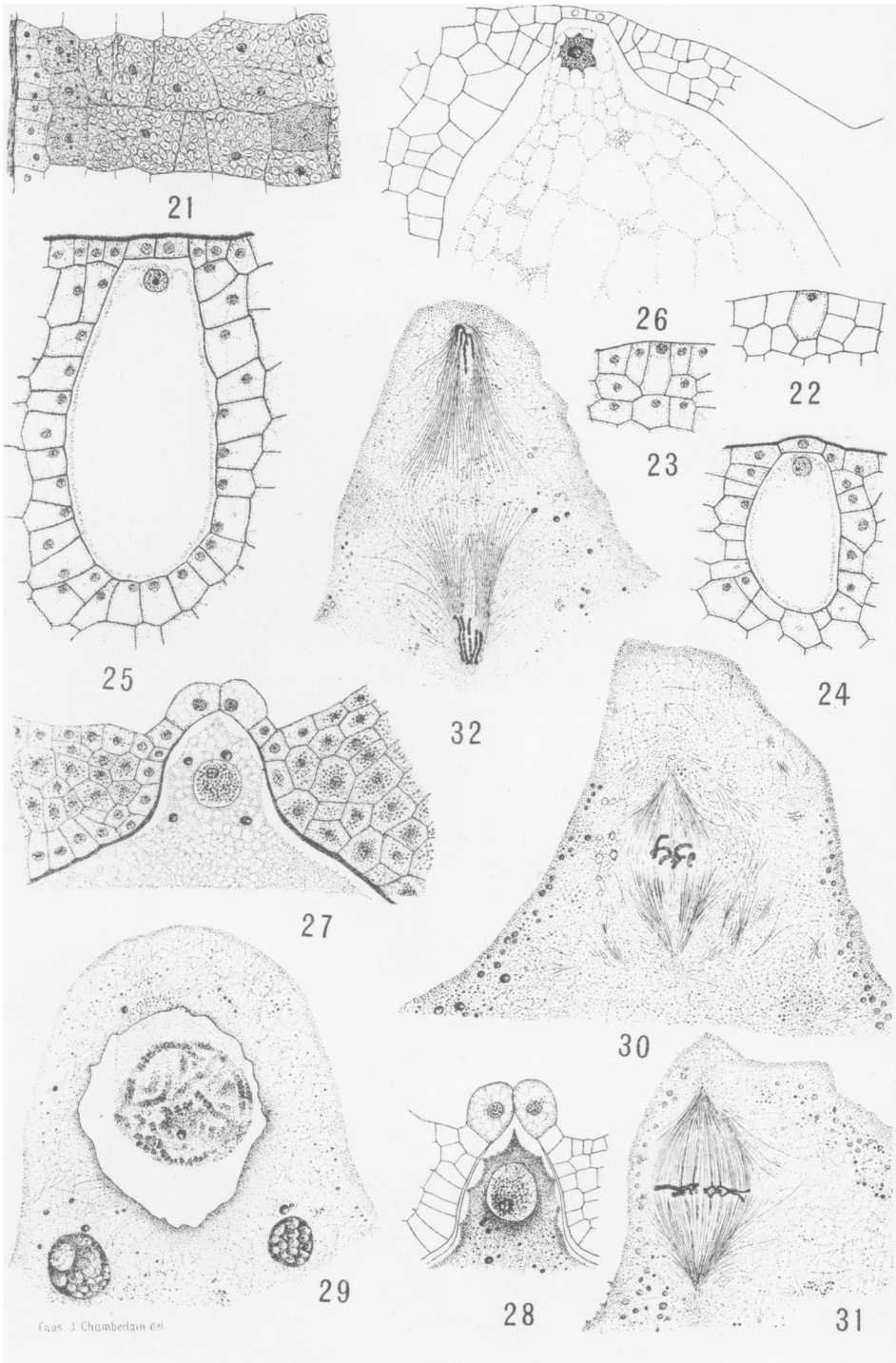
EXPLANATION OF PLATES XIII–XV.

(FIGS. 1–9 are text cuts.)

FIG. 10. Longitudinal section of ovule: *a*, abscission layer; *e*, endosperm; *i*, inner fleshy layer of integument; *ib*, bundle of inner vascular system; *m*, micropyle; *n*, free portion of nucellus; *o*, outer fleshy layer of integument; *ob*, bundle of outer fleshy layer; *p*, basal papilla; *s*, stony layer of integument. ×2.



CHAMBERLAIN on DIOON



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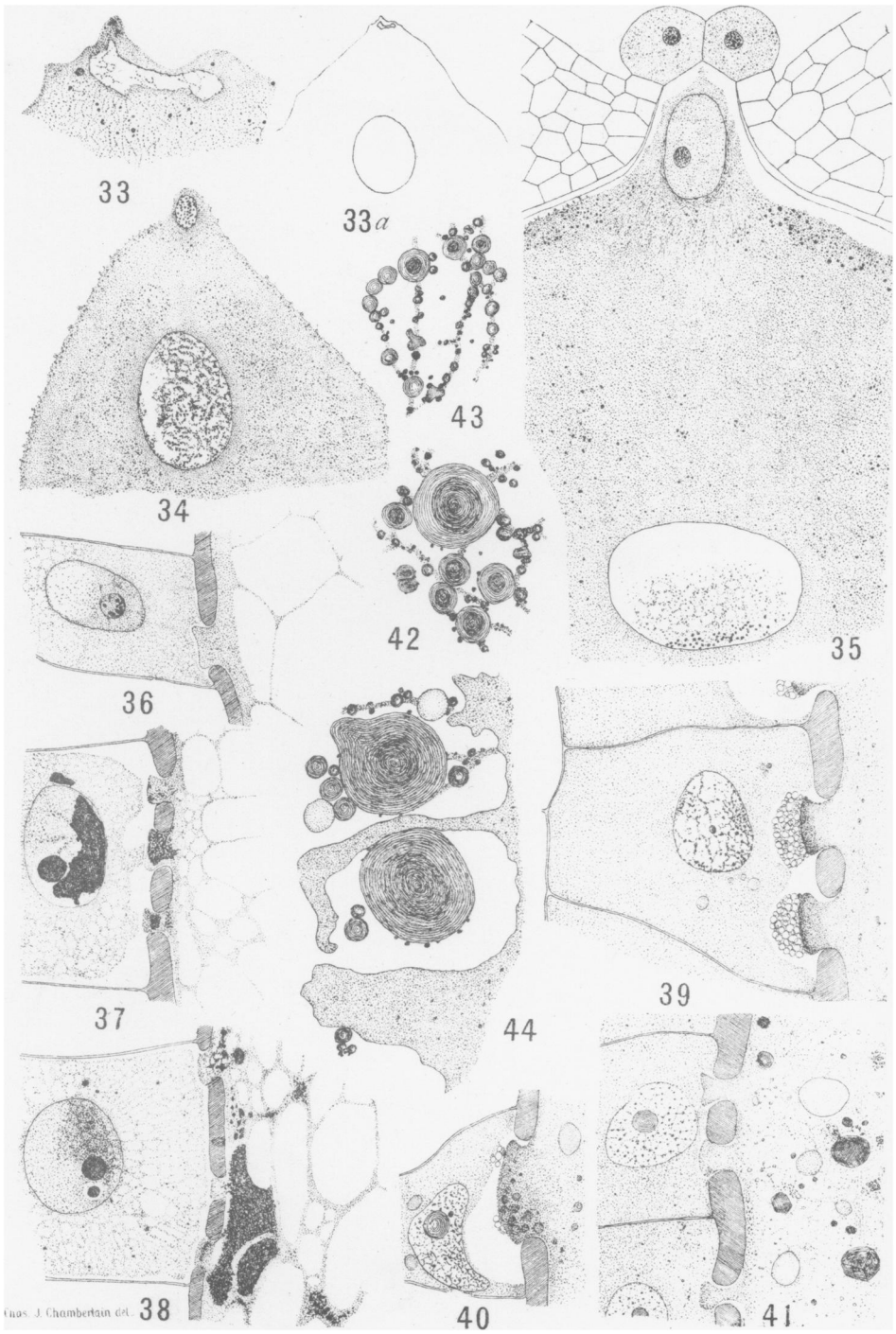

CHAMBERLAIN on DIOON

FIG. 11. Longitudinal section through integument and adnate portion of nucellus: *e*, epidermis; *el*, longitudinally elongated cells of stony layer; *et*, transversely elongated cells of stony layer; *ej*, endosperm jacket; *g*, endosperm; *i*, isodiametric cells of stony layer; *ib*, bundle of inner vascular system; *if*, inner fleshy layer of integument; *il*, inner layer of longitudinally elongated cells of stony layer; *n*, adnate portion of nucellus; *ob*, bundle of outer vascular system; *of*, outer fleshy layer of integument; *p*, parenchyma cells of outer fleshy layer; *s*, stony layer; *t*, cells containing tannin. ×2.

FIG. 12. Section showing endosperm (*e*), megaspore membrane (*m*), and endosperm jacket (*ej*). ×350.

FIG. 13. Cell of endosperm jacket showing one normal nucleus and a free spirem of another nucleus. ×350.

FIG. 14. Longitudinal section through abscission layer; *t*, cells containing tannin. ×112.

FIG. 15. Section of archegonia early in December, showing endosperm somewhat raised over the archegonia. Natural size.

FIG. 16. Section of archegonia showing beginning of archegonial chamber. Natural size.

FIG. 17. Section of archegonia just after formation of ventral canal nucleus. ×5.

FIG. 17*a*. The same, natural size.

FIG. 18. Transverse section of megaspore membrane. ×900.

FIG. 19. Section of lateral endosperm at a period when archegonium initials are forming at the apex; 280 cells in entire periphery of section. ×88.

FIG. 20. Similar section at a later stage, 780 cells in periphery; the two rows of cells marked *a* correspond to the single row *a* of *fig. 19*. ×88.

FIG. 21. Peripheral portion of endosperm at period of germination. ×88.

FIGS. 22–23. Archegonium initials. ×88.

FIG. 24. Archegonium, Dec. 21. ×88.

FIG. 25. Archegonium early in January. ×88.

FIG. 26. Upper portion of archegonium. ×88.

FIG. 27. Upper portion of archegonium, March 7. ×88.

FIG. 28. Upper portion of archegonium, May 13, over two months after cone was picked. ×88.

FIG. 29. Nucleus of central cell and nucleolus-like bodies in the cytoplasm. ×350.

FIGS. 30–32. Stages in the division of the nucleus of the central cell to form the ventral canal nucleus and egg nucleus. ×350.

FIG. 33. Ventral canal nucleus. ×350.

FIG. 33*a*. Upper portion of egg, showing ventral canal nucleus and egg nucleus. ×22.

FIG. 34. Ventral canal nucleus and egg nucleus, April. ×41.

FIG. 35. Neck, ventral canal nucleus, and egg nucleus. ×41.

FIG. 36. Portion of jacket cell and egg, showing haustorium, March 9. ×800.

FIG. 37. Jacket and haustoria, March. ×800.

FIG. 38. Porton of egg and jacket cell just after a discharge. ×800.

FIG. 39. Jacket cell and egg showing accumulation of globules over the haustoria. ×800.

FIG. 40. Similar haustorium with the globules passing into it. ×800.

FIG. 41. Jacket cells and haustoria when the egg is nearly ready for fertilization. ×800.

FIGS. 42–43. Details of structure of the egg nucleus. ×1300.

FIG. 44. Small portion of the egg nucleus showing the very irregular outline of the nuclear membrane. ×1300.